Helicobacter Pylori Different Virulence Genes and the Risk of Gastric Cancer in Iranian Patients

Maryam Kianmehr

Qom Islamic Azad University: Islamic Azad University of Qom

Ahmad Hormati (✉ hormatia@yahoo.com)

Qom University of Medical Sciences and Health Services <https://orcid.org/0000-0003-4321-874X>

Mohsen Zargar

Qom Islamic Azad University: Islamic Azad University of Qom

Roohollah Fateh

Qom University of Medical Sciences and Health Services

Razieh Nazari

Qom Islamic Azad University: Islamic Azad University of Qom

Research article

Keywords: Gastric cancer, virulence gene, *Helicobacter pylori*

Posted Date: December 16th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-126211/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Some disease-specific *Helicobacter pylori* virulence genes can be used for predicting the outcome of diseases. Thus, the current study aimed to explore the frequency of the *vacA*, *cagA*, *sabA*, *dupA*, *babA*, *oipA*, and *iceA1* genes in *H. pylori* isolates, and to determine whether any association exists between the expression of these genes and gastric cancer (GC).

Methods: *H. pylori* isolates were collected from individuals with digestive diseases. The *cagA*, *vacA*, *dupA*, *sabA*, *babA*, *oipA*, and *iceA1* genes were determined by PCR. The qRT-PCR was used for expression analysis of the tested genes.

Results: The presence of the *cagA*, *vacA*, *dupA*, *sabA*, *babA*, *oipA* and *iceA1* genes in *H. pylori* isolates were 41.3%, 95.5%, 31.0%, 93.1%, 55.1%, 82.7%, and 62.0%, respectively. The *cagA*, *dupA*, and *babA* expression in the GC patients was statistically higher than that of the control group ($P < 0.05$).

Conclusions: Our results indicated a high diversity and frequency of *H. pylori* virulence genes among individuals with gastric diseases in the Iranian population. The *cagA*, *dupA*, and *babA* expression were significantly higher in the GC patients, thus, it may suggest that screening of these genes may help identify peoples at higher risk for GC.

Background

Gastric cancer (GC) is a global concern and remains the third leading cause of mortality from cancer worldwide [1]. Positive serologic tests for *Helicobacter pylori* is among the main causes of GC, accounting for approximately 89% of GC cases worldwide [1–3]. The relationship of *H. pylori* infection and several extra-intestinal diseases, such as migraine, Alzheimer's, and mild cognitive impairment, has also been addressed [4–8].

Earlier surveys indicated that those with chronic infection with *H. pylori* were more likely to develop GC [9–11]. Moreover, the World Health Organization and the International Agency for Research on Cancer (IARC) categorized *H. pylori* as a Group 1 carcinogen for humans [12].

Among the individuals infected with *H. pylori*, only 1–3% progress to GC [13]. Some *H. pylori* virulence genes may play an essential role in the progress of gastric diseases. The strongest candidates include the outer inflammatory protein (*oipA*), the sialic acid-binding adhesin (*SabA*), the vacuolating cytotoxin (*vacA*), the blood group antigen-binding adhesin (*babA*), the cytotoxin-associated antigen A (*cagA*), duodenal ulcer promoting gene (*dupA*), and induced by contact with epithelium gene (*iceA*) [14–20]. These virulence genes may lower the threshold for neoplastic alteration by dysregulating the host intracellular signaling pathways [13].

In Iran, the rate of *H. pylori* infection is very high lies between 60% and 90% in different ages and geographic regions [21]. Likewise, the majority of *H. pylori*-infected subjects are *cagA*-positive strains that

are related to a greater risk of GC in some Iranian populations [21]. Despite the high prevalence of *cagA* positive *H. pylori* in Iran, its contribution to excess cancer risk is unclear. Furthermore, limited studies in Iran have concurrently investigated the relationship to GC of these virulence-related genes. Thus, in the current study, we examined the association of *cagA*, *vacA*, *dupA*, *sabA*, *babA*, *oipA*, and *iceA1* genes of *H. pylori* with GC.

Methods

Study population, samples, and culture

Gastric biopsies for isolation of *H. pylori* were taken from subjects with digestive diseases at a teaching hospital in Qom, Iran in the year 2018. Eleven biopsy specimens including four specimens of the greater and lesser curvature of the antrum and four specimens of small and large curvature of the gastric body and two specimens of the duodenum (5 biopsies for histopathological tests and 5 biopsies for culture and molecular tests) and one specimen for testing Rapid urease RUT was taken from each patient.

The culture method was performed as described by Dabiri et al, previously [22]. Briefly, each specimen was placed into brucella Agar with 10% defibrinated sheep blood containing the supplement (5 mg/l amphotericin B, 5 mg/l trimethoprim, 10 mg/l vancomycin). The plates were placed at 37 °C (CO₂ 10%, N₂ 85%, O₂ 5%) and were studied after 5 days of incubation. Urease, catalase, oxidase tests were used for confirmation of *H. pylori* isolates. The colonies of *H. pylori* were further used for molecular analysis.

Commercial Rapid Urease Test

The RUT was done as described by the instructions of the manufacturer (HelicotecUT® Plus, Taiwan). The tests were recorded and observed within 1 hour. The color changed from yellow to pink indicated a positive RUT.

Molecular Identification Of *H. Pylori*

DNA was extracted using a commercial kit (Bio Basic, Canada). For molecular confirmation of *H. pylori* isolates, PCR for *16SrRNA* and *glmM* genes using specific primers was performed based on published papers [23].

PCR assays for virulence genes (*cagA*, *vacA*, *dupA*, *sabA*, *babA*, *oipA*, and *iceA1*)

PCR was carried out according to the study of Dabiri and colleagues [22]. The primers sequences used for *vacA*, *cagA*, *dupA*, *sabA*, *babA*, *oipA*, and *iceA1*, and the expected product lengths are shown in Table 1. Negative control (Double distilled water) and positive control (*H. pylori* DNA 26695 and J99) reactions were used in the PCR run.

Table 1
Primer sequences of the tested genes *Refers*.

Genes	Primers (5 – 3)	Product (bp)
<i>glmM</i>	Forward - AAGCTTTTAGGGGTGTTAGGGGTTT Reverse - AAGCTTACTTTCTAACACTAACGC	294
<i>16S rRNA</i>	Forward - CTGGAGAGACTAAGCCCTCC Reverse - ATTACTGACGCTGATTGTGC	110
<i>cagA</i>	Forward - TTGACCAACAACCACAAACCGAAG Reverse - CTTCCCTTAATTGCGAGATTCC	183
<i>vacA</i>	Forward - ATGGAAATACAACAAACACAC Reverse - CAACAATGGCTGGAATGAT	136/163
<i>iceA1</i>	Forward - GTGTTTTTAACCAAAGTATC Reverse - CTATAGCCASTYTCTTTGCA	247
<i>babA</i>	Forward - CACGATCAGTTCAAAAG Reverse - TTRATGAGCGTGCTCGCTTGCG	138
<i>dupA</i>	Forward - CAAAGGAACACAACAAACCT Reverse - CGATATAGGCAAACATTATAGGATG	84
<i>oipA</i>	Forward - GTTTTTGATGCATGGGATTT Reverse - GTGCATCTCTTATGGCTTT	401
<i>sabA</i>	Forward - CCAACAACATTGAGCTGGTC Reverse - TTGCAARATRGGTATCATCG	593

Rna Extraction And Qrt-pcr

RNA was extracted using an RNA extraction kit (BioFACT™ Total RNA Prep Kit, Korea) according to the manufacturer's instructions.

The qRT-PCR was used for expression analysis of *H. pylori* pathogenicity genes. The PCR conditions and primer sequences for the *16srRNA*, *cagA*, *vacA*, *dupA*, *sabA*, *BabA*, *oipA*, and *iceA1* genes have been described previously [23–30]. A cDNA synthesis kit (BioFACT™ 2-Step 2X RT-PCR Pre-Mix (Taq) Kit, Korea) was used for cDNA synthesis. Amplification was done using 40 cycles, each cycle consisting of 94 °C for 15 s, 55 °C (*cagA* and *dupA*), 56 °C (*16srRNA* and *SabA*), 53 °C (*babA*), 52 °C (*oipA*), 51 °C (*vacA*) or 49 °C

(*iceA1*) for 30 s, and 72 °C for 25 s. The reactions were carried out using an ABI step ONE Plus thermal cycler. A 2% agarose gel was used for the analysis of products.

Statistical Analysis

Data analysis was carried out using Statistical Package for the Social Sciences software (SPSS version 23.0) and Graph Pad Prism software version 8.0.2. A Chi-square test was used for the analysis of categorical data. ANOVA statistical test and Tukey post hoc test were applied for quantitative analysis. P values of less than 0.05 were considered statistically significant.

Results

In this study, 190 patients with digestive disorders were enrolled. Among them, 13 were excluded from the study due to the unavailability of demographic information. Based on histological and endoscopic findings, patients were classified into three groups: 91 people without ulcer (NUD = non-ulcer dyspepsia), 55 with peptic ulcer disease (PUD), and 31 with GC. According to the RUT, 51 (28.8%) tissue samples were positive for *H. pylori* and 126 (71.2%) specimens were negative. In the histology method, 40 (22.6%) biopsy specimens were positive and 137 (77.4%) specimens were negative. Likewise, in the culture method, *H. pylori*-positive and -negative samples were 42 (23.7%) and 135 (76.3%) respectively. Among the specimens with a positive culture, 13 samples were excluded from the study due to a low number of bacteria and insufficient amounts of DNA and RNA for molecular tests. Finally, 29 isolates of *H. pylori* remained for further analysis (Fig. 1).

The patients with positive culture were assessed for the relation of gender, age, ethnic group, history of smoking, and alcohol intake with the severity of illness (Table 2). No statistically significant difference was observed between these variables and gastroduodenal diseases. However, the age groups of > 60 years had an increased rate of GC disease.

Table 2
Distribution of 29 patients with different clinical outcomes, according to age, gender, ethnic group, history of smoking, and alcohol intake.

Parameters	Non-ulcer (N = 13)	Peptic ulcer (N = 8)	GC (N = 8)	P-value
Gender	6 (46%)	4 (50%)	7 (87.5%)	0.15
Male	7 (54%)	4 (50%)	1 (12.5%)	
Female				
Age	0	0	0	0.07
≤ 20	13 (100%)	8 (100%)	0	
40–60	0	0	8 (100%)	
> 60				
Ethnic group	12 (92.3%)	7 (87.5%)	7 (87.5%)	0.90
Iranian	1 (7.7%)	1 (12.5%)	1 (12.5%)	
Afghan				
History of smoking	1 (7.7%)	2 (25%)	1 (12.5%)	0.45
Yes	12 (92.3%)	6 (75%)	7 (87.5%)	
No				
Alcohol intake	0	0	0	0.90
Yes	13 (100%)	8 (100%)	8 (100%)	
No				

Pcr Results Of The Tested Genes

The presence of the *cagA*, *vacA*, *dupA*, *sabA*, *babA*, *oipA*, and *iceA1* genes was investigated in patients with a positive culture for the *H. pylori*. There was no statistical relationship between these genes and clinical Consequences (Table 3). The *cagA* and *vacA* genes were detected in 41.3% (12/29) and 96.5% (28/29) of the patients, respectively. Similarly, to *vacA*. *sabA*, and *oipA* were found more commonly in patients with *H. pylori*. Of the 29 *H. pylori*-infected samples, *dupA*, *babA* and *iceA1* were detected in 31.0% (9/29), 55.1% (16/29), and 62.0% (18/29), respectively. As shown in Table 3, *cagA*, *dupA*, and *babA* were most commonly found in the GC patients, whereas *sabA*, *oipA*, and *iceA1* were most commonly found in the Non-ulcer patients ($p > 0.05$). Although, the *vacA* gene was frequently found in all patient groups; there was no significant difference among the investigated patient ($p > 0.05$) (Table 3).

Table 3
The *cagA*, *vacA*, *DupA*, *SabA*, *BabA*, *OipA*, and *iceA1* status of *H. pylori* isolates obtained from 29 patients with different clinical outcomes.

Genes	Non-ulcer (N = 13)	Peptic ulcer (N = 8)	GC (N = 8)	P-value
<i>cagA</i>	3 (23%)	4 (50%)	5 (62.5%)	0.17
Positive	10 (77%)	4 (50%)	3 (37.5%)	
Negative				
<i>vacA</i>	13 (100%)	7 (87.5%)	8 (100%)	0.25
Positive	0	1 (12.5%)	0	
Negative				
<i>DupA</i>	3 (23%)	1 (12.5%)	5 (62.5%)	0.06
Positive	10 (77%)	7 (87.5%)	3 (37.5%)	
Negative				
<i>SabA</i>	13 (100%)	8 (100%)	6 (75%)	0.60
Positive	0	0	2 (25%)	
Negative				
<i>BabA</i>	6 (46.2%)	4 (50%)	6 (75%)	0.40
Positive	7 (53.8%)	4 (50%)	2 (25%)	
Negative				
<i>OipA</i>	12 (92.3%)	7 (87.5%)	5 (62.5%)	0.20
Positive	1 (7.7%)	1 (12.5%)	3 (37.5%)	
Negative				
<i>iceA1</i>	9 (69.2%)	5 (62.5%)	4 (50%)	0.60
Positive	4 (30.8%)	3 (37.5%)	4 (50%)	
Negative				

H. Pylori Virulence Gene Expression

Expression of the Virulence-associated genes *cagA*, *vacA*, *dupA*, *sabA*, *babA*, *oipA*, and *iceA1* was detected using qRT-PCR. Expression was calculated based on the cycle threshold for the gene of the target relative to that for the *16srRNA* (control group). As shown in Fig. 2A, *cagA* expression in the GC group was 2336-fold higher than that of the control group, which was statistical significance ($P < 0.0001$). Likewise, *dupA* and *babA* expressions in the GC group were statistically 378- and 3.4-fold higher than that

of the control group, respectively ($p < 0.05$) (Fig. 2B, C). On the contrary to the *cagA*, *dupA*, and *babA*, the expression of *vacA* and *sabA* genes in the GC group were significantly 1.6- and 4.5 times lower than that of the control group, respectively ($p < 0.05$). For *oipA* and *iceA1* genes, there was no significant difference between the GC and control groups ($p > 0.05$).

Discussion

In the current study, we indicated the association between virulence genes of *H. pylori* and the GC. *H. pylori* infections are frequently asymptomatic and only a few individuals develop GC [31]. It has been indicated that some *H. pylori* virulence genes may play a significant role in the progress of post-*H. pylori* infection disorders such as GC.

In the previous studies, *cagA* and *vacA* of *H. pylori* were shown to have a significant association with peptic ulcers and GC [32–36]. Nevertheless, other studies have not established this association, specifying geographic variation [37]. Based on our analysis, the *cagA* gene was observed in 41.3% of the *H. pylori*-infected patients. The current reports are in comparison with earlier reports that show the *cagA* gene was present in about 65% of *H. pylori* isolates obtained from Asian and Western peoples [37–39]. The *cagA*-positive isolates of *H. pylori* have been also reported to be associated with gastric mucosal atrophy and GC [35, 40]. In a meta-analysis study, it was shown that patients infected with *cagA*-positive *H. pylori* isolates have an increased risk for GC [32]. Similarly, the subsequent analysis showed that infection by *cagA*-positive *H. pylori* isolates can increase the risk of GC [35]. Gohardani *et al.* showed that the expression of *cagA* was higher in Iranian patients with peptic ulcers compared to those with non-ulcer dyspepsia [41]. However, in studies conducted in Korea and Japan, there was no significant association between the *cagA* gene and the severity of gastroduodenal diseases [42, 43]. According to recent studies, the diversity of *cagA* types may explain such discrepancy [37].

The expression of *babA* and *dupA* genes were also significantly associated with GC in the current study.

Gerhard *et al.*, and Hocker *et al.* revealed that *H. pylori* strain to carry *babA* was associated with the highest risk of the ulcer [44, 45]. In our results, however, there was not any association between *babA*- and *dupA*-positive *H. pylori* isolates and the development of GC, even though the expression of *babA* and *dupA* genes were also significantly associated with GC.

Our findings were inconsistent with the *dupA* gene being a marker for intestinal metaplasia and GC. In this survey, there was not any association between the presence of *dupA* and GC. Hong Lu *et al.* indicated that *dupA* was associated with a reduced risk for GC and an increased risk for duodenal ulcers [46]. A study in Brazilian adults also described that the existence of *dupA* was statistically lower in patients with GC [47]. However, Argent *et al.*, reported that the *dupA*-positive *H. pylori* strains were more frequent in GC patients than duodenal ulcers (71% vs. 50%) [48]. The difference between these studies may be related to differences in the descriptions of geographic variations of circulation strains or patient groups.

In the current study, the *vacA* gene was present in 96.5% of *H. pylori* isolates, but no significant association between this gene and GC was observed. Likewise, in Asian studies, the existence of *vacA* was not statistically associated with GC [42, 43].

The *iceA1* gene was not associated with GC in this study. However, some reports have proposed a reverse correlation [37, 38]. Two distinct allelic variants of *iceA1* exist, in which *iceA1* has been suggested to be associated with gastric disease [37].

The *oipA* gene was existing in 82% of *H. pylori* isolated in the current which was following the previous studies [49, 50]. Kudo et al. and Yamaoka et al. also identified the *oipA* gene from 30% and 45.9% of *H. pylori* isolates, respectively [51, 52]. Similar to our result, Shao *et al.*, declared that no relationship exists between the *oipA* gene and gastrointestinal diseases [53]. Overall, the association between the *oipA* gene and GC needs further investigation.

In our study, the expression of some virulence genes of *H. pylori* was significantly higher in GC patients. Thus, it has been revealed that the eradication of *H. pylori* in patients with early GC would prevent the progress of new cancer [54].

Conclusion

Our finding indicated a high diversity and frequency of *H. pylori* virulence genes in GC patients in the Iranian population. Although there was not a significant association between different virulence genes with the clinical outcomes, the *cagA*, *dupA*, and *babA* expression in the GC group was significantly higher than that of the control group. Thus, it may suggest that screening of these genes may help identify populations at higher risk for GC.

Abbreviations

Helicobacter pylori (*H. pylori*)

Outer inflammatory protein (*oipA*)

Sialic acid-binding adhesin (*SabA*)

Vacuolating cytotoxin (*vacA*)

Blood group antigen-binding adhesin (*babA*)

Cytotoxin-associated antigen A (*cagA*)

Duodenal ulcer promoting gene (*dupA*)

Induced by contact with epithelium gene (*iceA*)

Gastric Cancer (GC)

Polymerase chain reaction (PCR)

Real-time quantitative PCR (qRT-PCR)

International Agency for Research on Cancer (IARC)

Rapid urease test (RUT)

Complementary DNA (cDNA)

Deoxyribonucleic Acid (DNA)

Ribonucleic Acid (RNA)

Non-ulcer dyspepsia (NUD)

Peptic ulcer disease (PUD)

Declarations

Ethics approval & consent to participate

The study protocol was approved by the ethics committee of the School of Medicine, Tehran Azad University of Medical Sciences (No: IR.IAU.TMU.REC.1397.310). Writing informed consent, obtained from all patients.

Consent for publication

Not applicable.

Availability of data and material

All data and materials are available from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

This article was extracted from the Ph.D. thesis of Maryam Kianmehr in Biology-Microbiology from the Faculty of Science of the Islamic Azad University of Qom Branch.

Authors' contributions

[MK], writing and original draft preparation. [AH], correspondence(s). [MZ] and [RF], designing, investigation and writing. [RN], Review and revise of the manuscript. All authors have read and approved the manuscript.

Acknowledgements

The authors thank S Ahmadpour, from the Qom University of Medical Sciences, Qom, Iran, for the editing assistance.

References

1. Thrift AP, El-Serag HB. Burden of gastric cancer. Clin Gastroenterol Hepatol. 2020;18(3):534–42.
2. Lochhead P, El-Omar EM. Helicobacter pylori infection and gastric cancer. Best practice research Clinical gastroenterology. 2007;21(2):281–97.
3. Plummer M, et al. Global burden of gastric cancer attributable to Helicobacter pylori. International journal of cancer. 2015;136(2):487–90.
4. Akbari N, et al. Migraine, dyspepsia, and Helicobacter pylori: Zeroing in on the culprit. Iranian journal of neurology. 2019;18(1):19.
5. Hormati A, et al., *Migraine and gastric disorders: Are they associated?* Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 2019. 24.
6. Kountouras J, et al. Relationship between Helicobacter pylori infection and Alzheimer disease. Neurology. 2006;66(6):938–40.
7. Kountouras J, et al. Association between Helicobacter pylori infection and mild cognitive impairment. European journal of neurology. 2007;14(9):976–82.
8. Sun J, et al. A Meta-Analysis of the Association between Helicobacter pylori Infection and Risk of Coronary Heart Disease from Published Prospective Studies. Helicobacter. 2016;21(1):11–23.
9. Leodolter A, et al., *Somatic DNA Hypomethylation in H. pylori-Associated High-Risk Gastritis and Gastric Cancer: Enhanced Somatic Hypomethylation Associates with Advanced Stage Cancer.* Clinical and translational gastroenterology, 2015. 6(4): p. e85.
10. Helicobacter, Group CC. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. Gut. 2001;49(3):347–53.
11. Pilotto A, Franceschi M. Helicobacter pylori infection in older people. World journal of gastroenterology: WJG. 2014;20(21):6364.
12. Organization WH. *Biological agents. Volume 100 B. A review of human carcinogens.* IARC monographs on the evaluation of carcinogenic risks to humans/World Health Organization, International Agency for Research on Cancer, 2012. 100(Pt B): p. 1–441.
13. Wang F, et al. Helicobacter pylori-induced gastric inflammation and gastric cancer. Cancer letters. 2014;345(2):196–202.

14. Shiota S, et al. *Helicobacter pylori* iceA, clinical outcomes, and correlation with cagA: a meta-analysis. *PloS one*. 2012;7(1):e30354.
15. Posselt G, Backert S, Wessler S. The functional interplay of *Helicobacter pylori* factors with gastric epithelial cells induces a multi-step process in pathogenesis. *Cell Communication Signaling*. 2013;11(1):77.
16. Kim JM, *H. pylori* virulence factors: toxins (*CagA*, *VacA*, *DupA*, *OipA*, *IceA*), in *Helicobacter pylori*. 2016, Springer. p. 77–88.
17. Su Y-L, et al. Combination of *OipA*, *BabA*, and *SabA* as candidate biomarkers for predicting *Helicobacter pylori*-related gastric cancer. *Scientific reports*. 2016;6:36442.
18. Praszkier J, Sutton P, Ferrero RL, *Virulence mechanisms of Helicobacter pylori: an overview*, in *Helicobacter pylori Research*. 2016, Springer. p. 57–87.
19. White JR, Winter JA, Robinson K. Differential inflammatory response to *Helicobacter pylori* infection: etiology and clinical outcomes. *Journal of inflammation research*. 2015;8:137.
20. Proença-Modena JL, Acrani GO, Brocchi M. *Helicobacter pylori: phenotypes, genotypes and virulence genes*. 2009.
21. Malekzadeh R, Derakhshan MH, Malekzadeh Z. Gastric cancer in Iran: epidemiology and risk factors. *Arch Iran Med*. 2009;12(6):576–83.
22. Dabiri H, et al. Prevalence of *Helicobacter pylori* *vacA*, *cagA*, *cagE*, *oipA*, *iceA*, *babA2* and *babB* genotypes in Iranian dyspeptic patients. *Microb Pathog*. 2017;105:226–30.
23. Lu J-J, et al. Comparison of Five PCR Methods for Detection of *Helicobacter pylori* DNA in Gastric Tissues. *J Clin Microbiol*. 1999;37(3):772–4.
24. Sicinschi LA, et al. Non-invasive Genotyping of *Helicobacter pylori* *cagA*, *vacA*, and *hopQ* from Asymptomatic Children. *Helicobacter*. 2012;17(2):96–106.
25. Fujimoto S, et al. *Helicobacter pylori* *BabA* expression, gastric mucosal injury, and clinical outcome. *Clin Gastroenterol Hepatol*. 2007;5(1):49–58.
26. Imagawa S, et al. *Helicobacter pylori* *dupA* and gastric acid secretion are negatively associated with gastric cancer development. *Journal of medical microbiology*. 2010;59(12):1484–9.
27. Mansour KB, et al. Prevalence of *Helicobacter pylori* *vacA*, *cagA*, *iceA* and *oipA* genotypes in Tunisian patients. *Annals of Clinical Microbiology Antimicrobials*. 2010;9(1):10.
28. Kato S, et al. *Helicobacter pylori* *sabA* gene is associated with iron deficiency anemia in childhood and adolescence. *PloS one*. 2017;12(8):e0184046.
29. Szkaradkiewicz A, et al. Expression of *cagA*, *virB/D* complex and/or *vacA* genes in *Helicobacter pylori* strains originating from patients with gastric diseases. *PloS one*. 2016;11(2):e0148936.
30. Avilés-Jiménez F, et al. In vivo expression of *Helicobacter pylori* virulence genes in patients with gastritis, ulcer, and gastric cancer. *Infect Immun*. 2012;80(2):594–601.
31. Goudarzi M, et al. Evaluation of antimicrobial susceptibility and integron carriage in *Helicobacter pylori* isolates from patients. *Gastroenterology Hepatology from bed to bench*. 2016;9(Suppl1):S47.

32. Matos JI, et al. *Helicobacter pylori* CagA and VacA genotypes and gastric phenotype: a meta-analysis. *Eur J Gastroenterol Hepatol*. 2013;25(12):1431–41.
33. Sugimoto M, Zali M, Yamaoka Y. The association of vacA genotypes and *Helicobacter pylori*-related gastroduodenal diseases in the Middle East. *European journal of clinical microbiology infectious diseases*. 2009;28(10):1227–36.
34. Sugimoto M, Yamaoka Y. The association of vacA genotype and *Helicobacter pylori*-related disease in Latin American and African populations. *Clin Microbiol Infect*. 2009;15(9):835–42.
35. Ghotaslou R, et al. Risk of gastric cancer in association with *Helicobacter pylori* different virulence factors: A systematic review and meta-analysis. *Microb Pathog*. 2018;118:214–9.
36. Fernández-Reyes M, et al. *Helicobacter pylori* pathogenicity and primary antimicrobial resistance in Northern Spain. *Eur J Clin Invest*. 2019;49(8):e13150.
37. Chomvarin C, et al. Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. *International Journal of Infectious Diseases*. 2008;12(1):30–6.
38. Podzorski RP, et al., *Analysis of the vacA, cagA, cagE, iceA, and babA2 genes in Helicobacter pylori from sixty-one pediatric patients from the Midwestern United States*. *Diagnostic microbiology and infectious disease*, 2003. 46(2): p. 83–88.
39. Ribeiro ML, et al., *Clinical relevance of the cag A, vac A and ice A genotypes of Helicobacter pylori in Brazilian clinical isolates*. *FEMS Immunology & Medical Microbiology*, 2003. **36**(3): p. 181–185.
40. Andreson H, et al. Association of cagA and vacA genotypes of *Helicobacter pylori* with gastric diseases in Estonia. *J Clin Microbiol*. 2002;40(1):298–300.
41. ZaraatgarGohardani HR, et al. The Significant Association of the dupA and cagA genes of *Helicobacter pylori* with Peptic Ulcer. *Journal of Human Environment Health Promotion*. 2018;4(4):159–63.
42. Kim SY, et al. Genotyping CagA, VacA subtype, IceA1, and BabA of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. *J Korean Med Sci*. 2001;16(5):579.
43. Maeda S, et al. Major virulence factors, VacA and CagA, are commonly positive in *Helicobacter pylori* isolates in Japan. *Gut*. 1998;42(3):338–43.
44. Gerhard M, et al., *Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin*. *Proceedings of the National Academy of Sciences*, 1999. **96**(22): p. 12778–12783.
45. Höcker M, Hohenberger P. *Helicobacter pylori* virulence factors—one part of a big picture. *The Lancet*. 2003;362(9391):1231–3.
46. Lu H, et al. Duodenal ulcer promoting gene of *Helicobacter pylori*. *Gastroenterology*. 2005;128(4):833–48.
47. Gomes LI, et al. Lack of association between *Helicobacter pylori* infection with dupA-positive strains and gastroduodenal diseases in Brazilian patients. *International journal of medical microbiology*. 2008;298(3–4):223–30.

48. Argent RH, et al. The presence of dupA in *Helicobacter pylori* is not significantly associated with duodenal ulceration in Belgium, South Africa, China, or North America. *Clinical infectious diseases*. 2007;45(9):1204–6.
49. Souod N, et al., *The study of the oipA and dupA genes in Helicobacter pylori strains and their relationship with different gastroduodenal diseases*. *Gastroenterology and hepatology from bed to bench*, 2015. 8(Suppl1): p. S47.
50. Douraghi M, et al. dupA as a risk determinant in *Helicobacter pylori* infection. *Journal of medical microbiology*. 2008;57(5):554–62.
51. Kudo T, et al. Correlation between *Helicobacter pylori* OipA protein expression and oipA gene switch status. *J Clin Microbiol*. 2004;42(5):2279–81.
52. Yamaoka Y, Reddy R, Graham DY. *Helicobacter pylori* virulence factor genotypes in children in the United States: clues about genotype and outcome relationships. *J Clin Microbiol*. 2010;48(7):2550–1.
53. Shao S-H, et al. Research progress on *Helicobacter pylori* outer membrane protein. *World Journal of Gastroenterology: WJG*. 2005;11(20):3011.
54. Uemura N, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med*. 2001;345(11):784–9.

Figures

Figure 1

Samples included in the study.

Figure 1

Samples included in the study.

A.

B.

C.

Figure 2

H. pylori virulence gene (cagA, dupA, and babA) expression.

A.

B.

C.

Figure 2

H. pylori virulence gene (*cagA*, *dupA*, and *babA*) expression.